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Applications of biotechnology and biochemical engineering for the improvement of *Jatropha* and Biodiesel: A review

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A B S T R A C T

Jatropha has drawn the attention of researchers in recent years due to its emergence as a highly suitable feedstock plant for Biodiesel production. Efforts have been made to improve the *Jatropha* plant material and Biodiesel production. Tissue culture, transformation and molecular marker based studies were conducted to improve the plant material. Biochemical engineering were undertaken to improve both the process and product of Biodiesel from *Jatropha*. Various approaches were taken up to improve the oil extraction process from seeds, enhance the quality and quantity of Biodiesel and analyze the engine performance of *Jatropha* Biodiesel. Most of the studies have been carried out in recent years due to the growing interest in energy security and global warming posed by fossil fuels. In this review we give details of various biotechnological and biochemical engineering efforts related to *Jatropha* and Biodiesel.

Contents

1. Introduction 5176
2. Biotechnological studies on *Jatropha* 5177
  2.1. Tissue culture studies 5177
  2.2. Transformation studies 5179
  2.3. Molecular studies on *Jatropha* 5179
  2.4. Characterization of *Jatropha* genes 5181
3. Biochemical engineering studies on *Jatropha* 5181
  3.1. *Jatropha* oil based studies 5181
    3.1.1. Utilization of *Jatropha* oil for electrification projects 5182
  3.2. Biodiesel production 5182
  3.3. Enzyme studies 5183
  3.4. Engine performance 5184
4. Conclusion 5184
  References 5184

1. Introduction

Jatropha is an important energy plant which has received more attention in recent years for its utilization in Biodiesel production. Since the prices of edible vegetable oils are higher than that of diesel fuel, non-edible crude vegetable oils like *Jatropha* are preferred as potential low priced Biodiesel [1]. A simple search in scientific publisher websites (www.springerlink.com and www.sciencedirect.com) with a key word "*Jatropha*" yielded results with more articles in recent years on *Jatropha* (Table 1). From this it is apparent that *Jatropha* attracted the attention of more researchers and as a result scores of reports are available in recent years. *Jatropha* has become an indispensable feedstock plant for the Biodiesel production. From the Family Euphorbiaceae, subfamily Platilobeae, the genus *Jatropha* possesses more than 70 species. Among them *Jatropha curcas*, *Jatropha pohliana* and *Jatropha gossypiifolia* produce seeds with high oil content [2]. *Jatropha* is emerging within academic fraternity, civil society and policy cir-

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Table 1
Year wise list of articles published on *Jatropha* (May 2010).

Springerlink		Sciencedirect	
Year	No. of articles	Year	No. of articles
2010-	85	2010	242
2000–2009	379	2009	295
1990–1999	33	2008	175
1980–1989	6	2007	112
1970–1979	2	2006	75
1960–1969	3	2005	77
1950–1959	1	2004	47
1940–1949	1	2003	29
1920–1929	1	2002	28
1880–1889	1	2001	26
1850–1859	1	1993–2000	158

Source: www.springerlink.com and www.sciencedirect.com.

cles as an interesting crop for strengthening the agrarian systems of resource-poor farmers. *Jatropha* has not been used traditionally as a crop; however scientification and biotechnologization processes have been initiated aimed at increasing its efficiency in cultivation [3].

Recently biofuels have been getting considerable attention because of global emphasis on reducing greenhouse gases and preserving the environment. The use of Biodiesel to (partially) replace fossil diesel also has a significant potential for reducing pollution and creating socioeconomic benefits for farmers [4]. Developing countries are facing a great threat to energy security and are also constrained with economic and environmental pressures for agricultural production. These problems forced the developing countries to produce the fuel above the earth by planting the energy plants like *Jatropha* instead of depending on fossil fuel imports. The anticipated diminution of petroleum reserves is also a reason for the exploration of alternative fuels like Biodiesel utilizing *Jatropha*. The utilization of edible food crops (corn, soya, etc.) for the production of biofuels is expected to create a short supply of food for human consumption. The utilization of non-edible and renewable crops such as *Jatropha* is expected to minimize this problem [1,5]. In addition to growing on degraded and marginal lands, this crop has special appeal, in that it grows under drought conditions and animals do not graze on it [6].

Even though *Jatropha* is a non-edible, oil yielding and drought tolerant plant, much suitable for Biodiesel production, it has a few limitations. *Jatropha* needs attention from biotechnologists and biochemical engineers as wild *Jatropha* cannot be used directly for the efficient Biodiesel production as it bears the following limitations.

1. *Jatropha* research has not yet identified varieties that are reliably high yielding.
2. The trees vary greatly in yield, oil content and oil quality.
3. It can survive poor growing conditions but, in the absence of sufficient water and nutrients, it has poor yields.
4. It takes 3–5 years to reach economic maturity, which is longer than annual oilseed crops.
5. Its toxicity prevents use of the seed cake for livestock feed, which otherwise would add significant value.
6. The toxicity may present a health risk to plantation workers, children and livestock.
7. *Jatropha* is susceptible to pests and diseases when grown as plantation monocrop.
8. *Jatropha* is not frost tolerant and cannot tolerate waterlogging.
9. *Jatropha* may act as a host for cassava diseases.
10. *Jatropha* oil is less suited to direct use as a mineral diesel substitute in cool climates due to its viscosity, which is higher than rapeseed oil.
11. *Jatropha* may become a weed problem in certain environments [6].

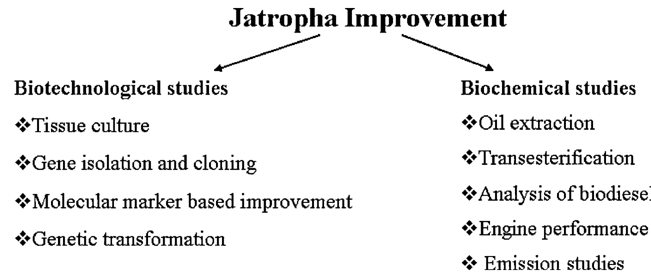


Fig. 1. Biotechnological and biochemical engineering studies for the improvement of *Jatropha*.

The popular belief that toxicity and insecticidal properties of *Jatropha* are sufficient deterrent for insects those cause economic damage in plantations. Several groups of insects have overcome this barrier. Particularly noteworthy is the insect order Heteroptera that has at least 15 species in Nicaragua, which can extract nutrients from physic nut. The key pest in Nicaragua was identified as *Pachycoris klugii*, *Agonosoma trilineatum* and *Scutellera nobilis* [7] and *Jatropha* is also attacked by other insects such as *Leptoglossus zonatus*, *Hypselonotus intermedius*, etc. The oil content of the seeds has been reduced by these insects [8]. In addition to insect damage, serious disease symptoms were also observed on a large number of *J. curcas* plants in various localities of Balrampur District, Uttar Pradesh, India during the rainy season of the year 2005. The symptoms consisted of mosaic from mild to severe, marked reduction in leaf size, rolling of leaf margins and puckering of leaf surface [9].

Due to the above listed limitations, *Jatropha* has been the subject of research in both developing and developed countries in recent years. Many of these limitations of *Jatropha* can be overcome by various biotechnological and biochemical engineering research (Fig. 1). The former can deal with the improvement of feedstock such as increase in the oil content by genetic engineering and Markers Assisted Selection (MAS), insect resistance and saline tolerance by introducing novel genes and alteration of fatty acid composition by metabolic engineering to improve the Biodiesel production. The latter can play a crucial role on downstream aspects of Biodiesel production including efficient oil extraction, developing of novel transesterification methods by chemical and enzymatic means and their optimization, improvement in the quality and quantity of Biodiesel, development and optimization of engines for Biodiesel and reduction of emission from engines.

Some excellent reviews are available on *Jatropha* [1,3,10–16]; however the present review is different from these and deals with the biotechnological and biochemical engineering studies conducted for the improvement of *Jatropha* plant and Biodiesel. This is a first such review covering wide spectrum of studies conducted on these fields.

2. Biotechnological studies on *Jatropha*

2.1. Tissue culture studies

Plant tissue culture (PTC) is a versatile tool for the biotechnological means of plant improvement. It has been applied increasingly in recent years for the development of transgenic plants where PTC is a vital prerequisite. PTC can also be utilized for the trait improvement of a plant by somaclonal variation. Although PTC is an old technique and many plant species have been subjected to this technique, *Jatropha* has got the attention of plant biotechnologists

Table 2
Tissue culture studies on *Jatropha*.

Explant	Type of culture	Medium	Phytohormones	References
Seven month old node	Clonal propagation	MS basal medium	22.2 μm BA + 55.6 μm adenine sulphate for inoculation 2.3 μM Kn + 0.5 IBA + 27.8 μm adenine sulphate for shoot proliferation 1.0 μm (IBA) for rooting	[23]
a. Node	*In vitro* propagation	MS basal medium	2.3–4.5 μm TDZ for primary culture; 4.4–8.9 μm BA for shoot multiplication	[21]
b. Leaf	Micropropagation	MS basal medium	8.9–44.4 μm BA + 4.9 μm IBA for adventitious shoot regeneration from leaf explants	
Leaves from seven month old plant	Somatic embryogenesis and regeneration	MS basal medium	9.3 μm Kn for callus induction and somatic embryogenesis 2.3 μm Kn and 1.0 μm IBA + 13.6 μm adenine sulphate for somatic embryo maturation and regeneration	[24]
Leaf	Direct shoot regeneration	MS basal medium	2.27 μm TDZ for induction of adventitious shoot buds	[28]
	Adventitious shoot induction	MS basal medium	BA + Kn + 0.5 μm IBA for multiplication of shoots	
Leaf and hypocotyls	Callus induction and cell suspension culture	MS basal medium	0.5 μm 2,4 D + coconut milk 2% (v/v) for callus induction form leaf 0.5 μm 2,4 D + coconut milk 2% (v/v) for callus induction form hypocotyls	[29]
Leaves at 3rd to 4th node from apex	Direct and indirect shoot organogenesis; elimination of endophytic bacterial contamination	MS basal medium	BA and IBA for direct shoot organogenesis and BA, 2,4-D and IAA for indirect shoot organogenesis, pulse treatment with TDZ Antibiotic augmentin for the elimination of endophytic bacterial contamination	[31]
Stems	*In vitro* regeneration and direct rooting.	MS basal medium	BA and Kn for the induction of shoot buds; BA and IAA for elongation of shoots; IBA for *in vitro* rooting 0.1–1.0 mg/l IBA and 3.5% sodium alginate matrix and polymerization medium containing 2.0% calcium chloride for direct rooting	[32]
Cotyledonary leaf	Direct regeneration	MS basal medium	TDZ shoot induction and Kn, BAP and NAA for shoot multiplication. Liquid medium containing IAA and NAA for rooting	[33]
Immature embryos	Callus induction and regeneration	MS basal medium	BA, IBA, casein hydrolysate, L-glutamine and $CuSO_4$ for callus induction and polyvinyl pyrrolidone, citric acid, Kn and IBA for regeneration. Trehalose for rooting	[34]
Leaf	Direct and indirect regeneration	MS basal medium	TDZ and IBA for callus induction and $CuSO_4$ for the regeneration.	[35]

only in recent years due to its potential as a feedstock for Biodiesel production. This technique has been applied to *Jatropha* by many researchers (Table 2). Earlier tissue culture studies performed on *Jatropha* have been reviewed by Sujatha et al. [11]. Different types of explants viz. node, leaf, apex and seeds were used for various types of *in vitro* studies. Murashige and Skoog's (MS) [17] basal salt medium was extensively used for tissue culture studies of *Jatropha*. Earlier studies include morphogenesis and plant regeneration [18], plant regeneration [19,20] and shoot bud proliferation from axillary nodes and leaf sections [21]. Efficient plant regeneration via shoot tip explant was also reported [22].

Use of benzyl adenine (BA) along with adenine sulphate promoted the shoot regeneration from nodal explants and addition of indole-3-butyric acid (IBA) induced shoots in *J. curcas* [23]. A protocol was developed by Jha et al. [24] for the somatic embryogenesis of *J. curcas*. MS medium containing kinetin (Kn) and IBA was used for somatic embryo induction and adenine sulphate was used for the conversion of somatic embryos to plantlets. Somatic embryogenesis is a most helpful technique for transgenic plant development. Establishment of efficient embryogenic cultures has become an integral part of plant biotechnology as regeneration of transgenic plants in most of the important crops is dependent on the formation of somatic embryos [25]. Large-scale production of SEs can also be applied to the production of artificial seeds [26]. A somatic embryogenesis pathway for plant regeneration has also been considered desirable for *Agrobacterium*-mediated transformation because it is assumed that SEs originate from single cells, thus the derived transgenic plants are unlikely to be chimeric and will originate from low numbers of transgene integration events [27]. The protocol developed by Jha et al. [24] for somatic embryogenesis may be utilized for successful development of transgenic plants in *Jatropha* in the near future.

Addition of thidiazuron (TDZ) with BA and IBA improved shoot regeneration from leaf disc explants; BA when added alone induced callus instead of shoot. Further multiplication of shoots was also achieved on MS basal medium supplemented with BA, Kn, indole-3-acetic acid (IAA) and gibberellic acid (GA_3) [28].

Coconut milk and 2,4-dichlorophenoxyacetic acid (2,4-D) were used by Soomro and Memon [29] along with BA and GA_3 for developing callus cultures in *J. curcas*. Two types of explants (leaf and hypocotyl) obtained from four day old seedling of *J. curcas* were used. They examined the influence of various compounds on growth of callus and found that 2,4-D gave the optimum response for efficient callus formation.

A study was undertaken by He et al. [30] to investigate browning of callus in *J. curcas*. They studied the changes in enzymatic activities (polyphenol oxidase (PPO) and peroxidase (POD)), pigments (chlorophylls and carotenoids) concentrations, differences in fatty acid composition, and cell structure differences in non-browning and browning callus derived from *J. curcas* hypocotyl explants. They found that PPO played more important role than POD in enzymatic callus browning.

Table 3
Transformation studies on *Jatropha*.

S. no.	Method of transformation	Plasmid	Promoter/selectable marker gene used	Promoter/selectable reporter gene used	Confirmation of transformation	References
1.	*Agrobacterium*-mediated	pCAMBIA2301	CaMV35S *hpt*	CaMV35S *GUS*	*GUS* assay, PCR and Southern blot	[37]
2.	Biolistic	pBI426	CaMV35S *npt-II*	CaMV35S *GUS*	*GUS* assay, PCR and Southern blot	[38]
3.	*Agrobacterium*-mediated	pCAMBIA2301	CaMV35S *npt-II*	CaMV35S *GUS*	*GUS* assay	[39]

Misra et al. [31] identified and controlled the endophytic bacterium of *J. curcas* which caused contamination during tissue culture. It caused contamination after 2–3 subcultures; the bacterium was identified as *Enterobacter ludwigii*. This bacterium was effectively controlled by supplementing augmentin in the culture medium. They obtained direct shoot organogenesis by employing BA and IBA and indirect shoot organogenesis by BA, 2,4-D and IAA in MS medium. They reported that a pulse treatment of TDZ and IBA for 5 days was necessary for shoot organogenesis in green compact callus.

Another protocol was developed recently for the shoot regeneration from stem explants of *J. curcas* [32]. Three elite genotypes (CSMCRI-I, CSMCRI-II and CSMCRI-III) were used in this study. Shoot buds were induced on MS medium containing BA and Kn; these shoot buds developed into shoots by adding BA and IAA in the regeneration medium. The shoots rooted in the medium containing IBA. In this study, authors also reported a protocol for direct rooting; this was achieved by dipping the base of shoots (4.0–5.0 cm) in MS medium supplemented with 0.1–1.0 mg/l IBA and 3.5% sodium alginate matrix and subsequently dropping in polymerization medium containing 2.0% calcium chloride.

In another study, MS medium containing TDZ induced shoot buds and Kn, BAP and NAA were used for shoot multiplication from cotyledonary node explants; they further proliferated by the addition of IAA and IBA. Rooting of shoots was achieved in liquid medium containing different concentrations and combinations of IAA and NAA for 4 days, followed by transfer to growth regulators-free half strength MS medium supplemented with activated charcoal [33]. Varshney and Johnson [34] used immature embryos of different stages for the callus induction and regeneration. They have used some additives viz. casein hydrolysate, L-glutamine and $CuSO_4$ on MS medium containing hormones. Efficient callus induction and plant regeneration were achieved on MS medium supplemented with polyvinyl pyrrolidone, citric acid, Kn and IBA. They have also used trehalose in half strength MS for root induction. More recently, Khurana-Kaul et al. [35] used different concentrations of $CuSO_4$ for the efficient regeneration of shoots from callus; they reported that significant improvement in shoot bud induction was observed when the concentration of $CuSO_4$ was increased to 10 times the normal MS level. These recent studies indicate that *in vitro* culture studies of *Jatropha* are emerging and these optimized PTC protocols may be utilized in the near future for efficient transformation.

2.2. Transformation studies

Although the transgenic technology is decade old and many transgenic plants have reached the field for commercial cultivation, *Jatropha* has got the attention of plant genetic engineers only in recent years due to its emergence as a biofuel plant. Only a few reports are available on transformation studies in *Jatropha* (Table 3).

A preliminary study on genetic transformation of *J. curcas* was conducted by Li et al. [36]; the details of this study were reviewed by Sujatha et al. [11]. A complete *Agrobacterium*-mediated transformation procedure for *J. curcas* was established for the first time in 2008 [37]. Bacterial strains LBA4404 and EHA105 were used for cocultivation and phosphinothricin was used for selection; about 55% of the cotyledon explants produced phosphinothricin-resistant calli. The transgenic nature of the transformants was demonstrated by the detection of ß-glucuronidase activity in the primary transformants and by PCR and Southern hybridization analyses. 13% of the total inoculated explants produced transgenic plants after approximately 4 months.

Two more studies had been reported in 2010 for the transformation of *J. curcas*. Purkayastha et al. [38] developed a direct DNA delivery system to shoot apices by particle bombardment. They had used the plasmid pBI426; it had *NPTII* gene for Kanamycin resistance for selection and *GUS* reporter gene to assay the transformation. Both these genes were put under the control of *CaMV53S* promoter. The plasmid coated microparticles were delivered to target explants using a biolistic *PDS*-1000/Helium system. They had also optimized various bombardment parameters, *i.e.*, microparticle size, bombardment pressure, target distance between stopping screen and target plate, and osmotic pretreatments and their duration for transient *GUS* expression. Transformation of *J. curcas* was confirmed by *GUS* staining, PCR analysis using *GUS* gene primers and Southern blot for *NPT-II* gene. Mazumdar et al. [39] developed a novel plant regeneration protocol by varying the age and orientation of explants and utilized the same protocol for the *Agrobacterium*-mediated transformation. They had used *Agrobacterium tumefaciens* strain EHA105 harbouring a binary vector pCAMBIA2301 which contained *GUS* and *NPTII* genes, both driven by CaMV 35S promoter. They confirmed that the *GUS* activity at the cut ends indicated the susceptibility of explants to *Agrobacterium*-mediated transformation.

Genetic transformation of *J. curcas* has been initiated only in recent times. Limited reports mentioned above alone are available. In these studies, only the expression of reporter or (and) marker gene(s) was tested. No transgenic *Jatropha* plant had been produced so far with the expression of functionally active foreign genes. Transgenic technology has been proved effective in overcoming the problems like insect damage, diseases and modification of fatty acid content of the oil [40,41]. This technology of crop improvement by inserting selective genes should be extended to *J. curcas* in the near future. This technology will help for the improvement of *Jatropha* in a more desirable way to serve as a best feedstock for the efficient Biodiesel production. Transgenic technology may offer some interesting possibilities in *Jatropha* improvement program including modification of lipid profile and resistance to biotic and abiotic stresses.

2.3. Molecular studies on Jatropha

Molecular marker based techniques are helpful for the MAS and improvement programs of plants. A molecular marker is defined as a particular segment of DNA that is representative of the differences at the genome level. Molecular markers may or may not correlate with phenotypic expression of a trait. Molecular markers offer numerous advantages over conventional phenotype based alternatives as they are stable and detectable in all tissues regard-

Table 4
Molecular marker based studies on *Jatropha*.

Type of molecular study	Variety/species analyzed	References
RAPD, ISSR and SCAR. Genetic variation	42 accessions of Indian *J. curcas* and a non-toxic Mexican variety.	[46]
RAPD and AFLP. Polymorphism	*J. curcas*, *J. glandulifera*, *J. gossypifolia*, *J. integerrima*, *J. multifida*, *J. podagrica* and *J. tanjorensis*	[44]
RAPP and AFLP. Genetic similarity	Toxic and non-toxic varieties of *J. curcas*	[45]
SPAR. Genetic diversity	*J. curcas* plants from different locations of India (NBRI, Lucknow, Bhubaneshwar, Lalkuan, Melli, Barapani and Itanagar)	[49]
ISSR–PCR, *Jatropha* species, genetic diversity, Biodiesel	3 accessions of *J. curcas* (TNMJ1, TNMJ22 and TNMJ23), *J. integerrima*, *J. podagrica*, *J. tanjorensis*, *J. gossyifolia*, *J. maheshwarii*, *J. mulifida*, *J. villosa* and *J. glandulifera*	[50]
RAPP and ISSR. Genetic diversity and interspecific variation	34 accessions of *Jatropha* sps. (*J. curcas*, *J. gossypifolia*, *J. glandulifera*, *J. integerrima*, *J. podagrica*, *J. multifida*, *J. villosa*, *J. villosa* var. *ramnadensis*, *J. maheshwarii*) and a natural hybrid, *J. tanjorensis*	[48]
AFLP. Polymorphism and genetic diversity	48 germplasm collections of *J. curcas* (UT1–11, SMHP, SDP CSM 1, 2, 3, 5, 9, 11, 12, SKNJ 2, 7, IJC 7, 8, 9, 11, OJC1-13, SNES 25, 34, 37, BAAS 30, 35, 38, 48, 53, 57, 61)	[47]

less of growth, differentiation, development, or defense status of the cell; they are not confounded by the environment [42]. The molecular marker techniques include Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeats (SSR), Amplified Fragment Length Polymorphism (AFLP), Sequence Characterized Amplified Region (SCAR), Inter Simple Sequence Repeat (ISSR) and Single Nucleotide Polymorphism (SNP). Some of these techniques have been successfully applied in *Jatropha* (Table 4) with a view to characterize and improve the plant. The details are discussed below.

An RAPD study was conducted to assess the genetic diversity of 12 species of *Jatropha* [43]. Twenty six random primers were used and 18 primers gave reproducible amplification banding patterns; UPGMA cluster analysis indicated three distinct clusters, the first comprising all accessions of *J. curcas* (TNMC 1, 3, 4, 5, 6), while the second included six species, viz. *Jatropha ramanadensis*, *J. gossypiifolia*, *Jatropha podagrica*, *Jatropha tanjorensis*, *Jatropha villosa*, *Jatropha integerrima* and *Jatropha glandulifera*; *J. integerrima* and *J. glandulifera* remained distinct and formed third cluster. This study may be helpful for collection, conservation and characterization of *Jatropha* genetic resources in future.

A phylogenetic relationship among seven species of *Jatropha*, namely *J. curcas*, *J. glandulifera*, *J. gossypifolia*, *J. integerrima*, *Jatropha multifida*, *J. podagrica* and *J. tanjorensis* was established by RAPD and AFLP techniques. The mean percentage of polymorphism was found to be 68.48% by RAPD and 71.33% by AFLP. The maximum relatedness was found between *J. curcas* and *J. integerrima*; this may be the reason for the success of inter hybrid crosses between these two species [44]. Another study was conducted at the same institute [45]; the authors used RAPD and AFLP markers to characterize toxic and non-toxic varieties of *J. curcas*. They analyzed 371 RAPD and 1442 AFLP markers; 56 (15.09%) RAPD and 238 (16.49%) AFLP markers were found specific to either of the varieties. Genetic similarity between nontoxic and toxic varieties was found to be 0.92 by RAPD and 0.90 by AFLP fingerprinting. This is the first report on molecular characterization of non-toxic and toxic *J. curcas*. The specific markers generated using RAPD and AFLP fingerprinting in this study will help to distinguish non-toxic from toxic varieties of *J. curcas* or vice versa and these markers may also be utilized in the near future for MAS and improvement of *Jatropha* based on the toxicity.

A molecular marker study was conducted by Basha and Sujatha [46] to find out the genetic diversity in 42 accessions of *J. curcas* grown in different regions of India and a non toxic variety from Mexico. The polymorphism was found to be 42.0% with 400 RAPD primers and 33.5% with 100 ISSR primers between accessions; this indicated the modest levels of genetic variation among the Indian germplasms. They also identified important ISSR markers for the differentiation of Indian accessions from Mexican genotype and two of the ISSR markers were converted to SCAR markers. Since the 'true centre of origin' of *J. curcas* has not been established so far, this type of study will pave the way for investigation of the genetic distinctness of this crop in the centre of origin and other regions where it is being introduced.

An AFLP analysis was performed by Tatikonda et al. [47] to assess the diversity in 48 elite germplasm collections of *J. curcas*. They used seven AFLP primer combinations which generated a total of 770 fragments with an average of 110 fragments per primer combination. A total of 680 (88%) fragments showed polymorphism in the germplasm analyzed of which 59 (8.7%) fragments were unique (accessions specific) and 108 (15.9%) fragments were rare. They found that the accessions coming from Andhra Pradesh, India were found to be diverse as these were scattered in different groups and accessions coming from Chhattisgarh, India showed occurrence of high number of unique/rare fragments.

Nuclear and organelle primers were also used for the characterization of *Jatropha* species occurring in India [48]. Thirty four accessions were used in this study; RAPD and ISSR primers were used for nuclear DNA and micro satellite primers were used for organelle DNA studies. It was concluded that characterization of advanced generation interspecific derivatives of *J. curcas* and *J. integerrima* cross, carried out in this investigation indicated ample scope for genetic enhancement of *J. curcas* through interspecific gene transfer. This study may be helpful to know the interspecific variations at genetic level.

Ranade et al. [49] developed a novel single-primer amplification reaction (SPAR) method for the genetic diversity study. They reported that The NBRI, Bhubaneshwar and Lalkuan accessions were more related to each other. The UPGMA tree clearly showed well-separated accession groups; NBRI, Bhubaneshwar, North East, Lalkuan and Outgroup. This may be an important tool and can be used for analyzing the diversity within the available collections to shortlist the parental lines for adaptability trials and further improvement of *Jatropha* plants.

Another study was conducted to assess the genetic diversity among 8 *Jatropha* species and 3 *J. curcas* accessions (TNMJ1, TNMJ22 and TNMJ23) using ISSR primers [50]. A few primers generated 100% polymorphic patterns. The UPGMA cluster analysis indicated 3 distinct clusters, the first comprising all accessions of *J. curcas*, while the second including 4 species viz *J. tanjorensis*, *J. gossypifolia*, *J. podagrica* and *Jatropha maheshwarii* and the third cluster including another four species (*J. villosa*, *J. multifida*, *J. integerrima* and *J. glandulifera*).

These studies indicate that molecular marker based studies on *Jatropha* have been vigorous and are on par with that of crop plants. These studies will help to develop new varieties of *Jatropha* soon

which can be a best feedstock variety for Biodiesel production adapting to all adverse effects of environment.

2.4. Characterization of Jatropha genes

A few studies were undertaken on characterization of various genes of *Jatropha*. In some of these studies, genes were isolated and cloned in a plasmid and expressed. Most of the studies also focused on the genes of fatty acid metabolism, because fatty acid profile is an important parameter affecting the Biodiesel production.

A group at College of Life Sciences, Sichuan University, China lead by Tong has been actively working on isolation and cloning of genes of *Jatropha*. In one study, cDNA of stearoyl-acyl carrier protein desaturase (SAD) was obtained by RT-PCR (reverse transcriptase) and RACE (Rapid Amplification of cDNA Ends) techniques from developing seeds of *J. curcas* [51]. This gene was also further cloned and expressed in *Escherichia coli*. Southern blot analysis indicated that the gene was a member of a small gene family. Northern blot analysis revealed that it was highly expressed in developing fruits of *J. curcas*. Another study was undertaken by the same group to clone SAD gene [52]. They had used the same techniques (RT-PCR and RACE) to clone a 1491 bp cDNA segment of SAD gene of *J. curcas*. This study confirmed that SAD had high level of homology both in nucleotide and amino acid sequences to other plant SADs.

In another lab, open reading frame (ORF) encoding curcin2 was cloned from total genomic and cDNA of *J. curcas* leaves, which were affected by drought, temperature stress and fungal infection, by PCR and RT-PCR amplifications. The ORF had 927 bp that enclosed a precursor protein of 309 amino acid residues. Antiserum to curcin recognized one band of 32 kDa on Western blot of the leaves treated by temperature stresses at 4 °C and 50 °C and by fungal infections of *Pestalotia funerea*, *Curvularia lunata*, *Gibberelle zeae*. Two bands of 32 kDa and 65 kDa were recognized on Western blot of the leaves treated with 10–40% Polyethylene glycol (PEG). In addition, the 32 kDa band was nearly the same as the molecular weight of curcin2. This finding suggested that the protein of 32 kDa was related to curcin2 [52]. The presence of this protein molecular marker under stresses may provide an experimental foundation to study the stress proteins in *J. curcas*.

3. Biochemical engineering studies on *Jatropha*

Biochemical engineering studies provide an opportunity to improve Biodiesel production from *Jatropha* oil and many such studies have been carried out by various researchers. In conventional processes, Biodiesel has been manufactured by the transesterification of oils with methanol in the presence of catalysts, such as alkali (KOH/NaOH) or their corresponding alkoxides [53]. Effects of different parameters like temperature, molar ratio of alcohol to oil, catalyst and reaction time, have been investigated by several researchers. Many works have also been carried out on engine performance and the emission characteristics with Biodiesel as a diesel engine fuel.

3.1. Jatropha oil based studies

Due to the increased demand for the utilization of *Jatropha* oil for Biodiesel production, a decorticator has been designed for the efficient extraction of oil from *Jatropha* seeds [54]. The major components of the machine include frame, hopper, decorticating chamber, concave sieve, rotating blades, discharge outlet and a vibrating separator with sieve to separate seed and shell. The authors suggest that because of its cheap cost to manufacture from locally available materials, it is likely to satisfy a niche market in India and other developing countries for the extraction of oil from *Jatropha* seeds.

In an oil extraction study on *J. curcas*, three phase partitioning (TPP) approach was used by Shah et al. [2]. The slurry was prepared by grinding *Jatropha* seed kernels (5 g/30 ml) in distilled water. The pH of the slurry was adjusted to the desired value and appropriate amount of ammonium sulphate was added and vortexed gently, followed by addition of appropriate amount of *t*-butanol. The slurry was then incubated for the three phase formation. The three phases were then separated by centrifugation at 2000 × *g* for 10 min after incubation at 25 °C for 1 h. The upper organic layer was collected and evaporated on rotary evaporator to obtain oil. They concluded that the novel approach of combining technique of TPP with enzyme pretreatment and sonication constituted an efficient procedure for obtaining oil from *Jatropha* seed kernels. The method developed in this study also took less time (about 2 h) [2].

Emil et al. [55] conducted a research to evaluate the physicochemical properties of seed oil extracted from the *Jatropha* seeds collected from different origin viz., Malaysia, Indonesia and Thailand to check their suitability for Biodiesel production. The physicochemical properties such as density, viscosity, percentage free fatty acid (FFA), iodine value, saponification value and peroxide value of the extracted *Jatropha* seed oil were evaluated. The authors recommended that these oils had the potential as a Biodiesel feedstock. Augustus et al. [56] conducted a study to find out the role of bioinduction on oil yield, polyphenol and hydrocarbon levels of *J. curcas*. They found that bioinduction had significantly increased the production of hydrocarbons. In the same study they had also tested various parameters of the oil viz. gross heat value, composition of fatty acids, etc. Treatment of plants with growth regulators significantly influenced the production of hydrocarbons.

Another study was conducted by Pramanik [57] with an objective to decrease the viscosity of *J. curcas* oil by dilution with diesel and to see the engine performance. He had prepared blends of varying proportions of *J. curcas* oil and diesel, analyzed and compared with diesel fuel; the effect of temperature on the viscosity of *Jatropha* oil and various blends was also studied. Based on properties and engine test results of his study it has been suggested that 40–50% of *Jatropha* oil can be substituted for diesel without any engine modification and preheating of the blends.

According to Tiwari et al. [58] *Jatropha* oil contains about 14% free fatty acid (FFA), which is far beyond the limit of 1% FFA level that can be converted into Biodiesel by transesterification using an alkaline catalyst. So they made an attempt to optimize the process parameters in pretreatment (esterification) and transesterification reactions for reduction of FFA of *Jatropha* oil below 1% and obtaining maximum yield of Biodiesel, respectively. They used Response Surface Methodology (RSM) based on Central Composite Rotatable Design (CCRD) to optimize the three important reaction variables, methanol quantity, acid concentration and reaction time for reduction of FFA content of the oil to around 1%. They found that the optimum combination for reducing the FFA of *J. curcas* oil from 14% to less than 1% was found to be 1.43% (v/v) H_2SO_4 acid catalyst, 0.28 (v/v) methanol-to-oil ratio and 88-min reaction time at a reaction temperature of 60 °C as compared to 0.16 (v/v) methanol-to-pretreated oil ratio and 24 min of reaction time at a reaction temperature of 60 °C for producing Biodiesel. They claimed that this process gave an average yield of Biodiesel with more than 99%.

Recently, Oliveria et al. [59] analyzed the oil of two species of *Jatropha* (*J. gossypiifolia* and *J. curcas*) for determining their potential as raw materials for Biodiesel production. They have analyzed the physical and chemical properties of the oils and the Biodiesel from these two species of *Jatropha*. They found that Biodiesel derived from the oils of *J. gossypiifolia* and *J. curcas* were in acceptable range for use as Biodiesel in diesel engines. This study confirmed the possibility of utilizing both these species of *Jatropha* as a feedstock for Biodiesel production.

Table 5
Biodiesel production from *Jatropha*.

S. no.	Source of the oil	Catalyst/method	Advantage	References
1.	*J. curcas*	Alkali; transesterification	98% yield of Biodiesel	[63]
2.	*J. curcas*	Alkali; transesterification, stripping/deodorization at 26 °C	Extraction of phorbol esters	[66]
3.	*J. curcas*	Alkali; transesterification; ultra-turrax, magnetic stirrer.	Extraction of phorbol esters	[4]
4.	Honge, *Jatropha* and sesame	Alkali; transesterification	Lower emissions of HC, CO and NOx	[67]
5.	*J. curcas*	Solid base catalyst, transesterification	Cheap and reusable catalyst	[68]
6.	*J. curcas*, sunflower, karanj, soybean and palm	Alkali; transesterification, effect of antioxidants	Good oxidative and low temperature properties	[69]
7.	*J. curcas*	Alkali; transesterification, two-step transesterification	90% yield of methyl ester	[70]
8.	*J. curcas*	Super critical methanol; transesterification	100% yield of esters with supercritical methanol	[71]
9.	*J. curcas*	Preesterification FFAs in the presence of an acid catalyst and esterification with methanol using an alkali catalyst	98% yield of Biodiesel within 20 min	[72]
10.	*J. curcas*	Supercritical hydrolysis and supercritical methylation	Highly pure (98%) Biodiesel	[73]
11.	*J. curcas*, *Azadirachta indica*, *Moringa oleifera*, *Aleurites trisperma*, *Ricinus communis* and *Aleurites moluccana*	Alkali; transesterification	Identification of best oil (*Jatropha*) source	[74]

Lipid profiling in developing seeds of *J. curcas* was performed by Annarao et al. [60] using high resolution NMR spectroscopy. Seed development stages from one week after fertilization to maturity were studied with respect to phenology, oil content, lipid profile and concentration of sterols. These were classified as stages I to VII; significant changes were observed in fatty acid profiles and synthesis of sterols. Ratio of linoleic to linolenic acid increased at first three stages while only linoleic acid alone was observed at stage IV onwards. This study may be helpful for improving the biosynthesis of triglycerides and reduce FFA content in the mature seeds of *Jatropha*.

3.1.1. Utilization of Jatropha oil for electrification projects

Seed oil of *J. curcas* has been successfully employed for power generation plants in 2006 at Ranidhera, Chhattisgarh, India. Winrock International India (WII) implemented this decentralized power generation plant fuelled by straight vegetable oil (SVO) from *J. curcas* [61]. Recently, Gmunder et al. [62] undertook a study to assess the environmental sustainability of the electrification project fuelled by straight *Jatropha* oil implemented in 2006. They conducted this study with a view to provide a scientific basis for policy decisions on electrifying remote villages with *Jatropha* oil. They also compared this *Jatropha* oil based plant with other electrification approaches such as photovoltaic (PV), grid connection and a diesel-fuelled power generator. They concluded that the *Jatropha*-based electrification in Ranidhera reduced greenhouse gas emissions over the full life cycle by a factor of 7 compared to a diesel generator or grid connection. Under these conditions, *Jatropha*-based electricity generation might be a useful alternative to other renewable electrification options, as the technology is very sturdy and can be maintained even in remote and highly underdeveloped regions [62]. From this study it is apparent that the *J. curcas* plant could be used as an energy crop for various sectors in addition to Biodiesel production. So policy makers should take steps for the proper utilization of waste land for the cultivation of *Jatropha* to increase the feedstock production to meet the demand of the energy sector especially in the developing world.

3.2. Biodiesel production

Many reports are available on Biodiesel production from *Jatropha* oil and various parameters were optimized (Table 5). The experimental conditions were optimized by Chitra et al. [63] at Tamil Nadu Agricultural University (TNAU), Coimbatore, India for improved Biodiesel production. Alkali-catalysed transesterification of *J. curcas* oil was performed to produce Biodiesel. NaOH was used as an alkaline catalyst. The important factors that affected the transesterification reaction were the amount of methanol, NaOH, reaction temperature and reaction time [64] and these were studied. They found that the maximum methyl ester yield of 98% was obtained using 20% methanol and 1.0% NaOH at 60 °C. The minimum reaction time required for maximum ester yield was found to be 90 min [64].

According to Goel et al. [65], presence of phorbol esters is a major constraint in the widespread acceptance of *Jatropha* as a source of Biodiesel, which, when consumed by man and animal, are toxic and are also cocarcinogens. This makes the oil unsuitable for food and feed applications. In view of the current debate of 'oil for food' versus 'oil for fuel', this toxicity is a potential advantage for *Jatropha*. *Jatropha* oil can be seen as 'technical oil', and therefore does not compete directly with the food markets. Studies on phorbol esters of *Jatropha* were conducted by Makkar et al. [66]. They quantified the phorbol esters in the fractions obtained at different stages of oil pre-treatment and Biodiesel production. They reported that Silica treatment did not decrease the phorbol esters, while stripping/deodorization at 26 °C at 3 mbar pressure with 1% steam injection completely degraded phorbol esters. Phorbol esters were not detected in stripped oil, fatty acid distillate, transesterified oil (Biodiesel) and glycerine. This work may contribute for the reduction of toxicity from the Biodiesel of *Jatropha*. Recently, Devappa et al. [4] assessed the effects of phorbol ester extraction on quality of both the residual oil and the Biodiesel produced from *J. curcas* oil. They applied two methods, one the use of an Ultra-turrax and the other, the use of a magnetic stirrer with an effective treatment time of 2 and 5 min, resulting in 80 and 78% extraction of phorbol esters, respectively. The Biodiesel prepared from both residual oils met European (EN 14214:2008) and American Biodiesel standard (ASTM D6751-09) specifications. The authors concluded that phorbol ester could be isolated in active forms for various applications by either of these two methods with a high yield and the residual oil can be processed to produce high quality Biodiesel.

In a study by Banapurmath et al. [67] the properties of Biodiesel prepared from Honge, *Jatropha* and sesame oils were determined; their combustion and emission characteristics were also studied on a four-stroke single-cylinder direct-injection compression ignition (CI) engine to check their feasibility as CI engine fuels. They

reported that the engine performance in terms of higher brake thermal efficiency and lower emissions (HC, CO, NOx) was observed with sesame oil methyl ester operation compared to methyl esters of Honge and *Jatropha* oil operation.

An efficient catalytic process using a solid base catalyst was developed by a group of Chinese scientists [68] for the production of Biodiesel from *J. curcas* seed oil. In this study, X/Y/MgO/c-Al_2O_3 exhibited high basicity, the best catalytic activity and mechanical strength for catalyzing the transesterification of *J. curcas* seed oil. The optimum reaction conditions were obtained by an orthogonal experiment. At the same time, catalyst recycling and regeneration were also investigated. The pilot plant tests were also carried out in a 100 L reaction vessel in this study. The scientists claimed that the identified catalyst was cheap and could be re-used for several runs without significant deactivation after regeneration.

A study was undertaken as per proposed National Mission on Biodiesel (India) on stability of Biodiesel from tree borne oil seeds viz. Sunflower oil, Karanj oil, *Jatropha* oil, Soybean oil and Palm oil. This study was conducted at Indian Oil Corporation Ltd., Faridabad, India [69]. According to this study, doping of antioxidant was required in order to improve the stability of Biodiesel and to make it acceptable to oil marketing companies. Further studies were carried out to compare efficiency of various antioxidants, especially for *Jatropha* Biodiesel and in comparison with other Biodiesels. *Jatropha* Biodiesel had poor oxidation stability with good low temperature properties. On the other hand, Palm Biodiesel had good oxidative stability, but poor low temperature properties. The combinations of *Jatropha* and Palm gave an additive effect on these two critical properties of Biodiesel.

A technique to produce Biodiesel from crude *J. curcas* seed oil having high free fatty acids (FFA) (15%) was developed by Berchmans et al. [70]. The high FFA level of *J. curcas* was reduced to less than 1% by a two-step pretreatment process. The first step was carried out with 0.60 (w/w) methanol-to-oil ratio in the presence of 1% (w/w) H_2SO_4 as an acid catalyst in 1-h reaction at 50 °C. After the reaction, the mixture was allowed to settle for 2 h and the methanol–water mixture separated at the top layer was removed. The second step was transesterified using 0.24 (w/w) methanol to oil and 1.4% (w/w) NaOH to oil as alkaline catalyst to produce Biodiesel at 65 °C. The second stage, alkali base catalysed transesterification process, gave 90% methyl ester yield.

Another study was conducted on transesterification process of *J. curcas* oil using super critical methanol in absence of a catalyst [71]. This was performed to investigate the effect of the operating variables on the conversion rate of the oil to esters, in order to optimize the conditions of the process. Different parameters involved in the process viz. temperature, pressure and molar ratio of alcohol to oil were investigated. The reaction products were also analyzed for their residual content of triglycerides, glycerol, monoglycerides, diglycerides, esters and free acids. They reported that 100% yield of esters was obtained using super critical methanol within four min only, at a temperature of 320 °C and under a pressure of 8.4 MPa. The authors recommended that supercritical methanol method had a high potential for transesterification of triglycerides to methyl esters or Biodiesel due to two major advantages; the reaction time was very short at reaction temperature of 320 °C and the separation of the products as well as the byproducts was achieved efficiently [70].

In order to overcome the problem associated with high content of FFA mentioned in Section 3.1 [58], a two-step process consisting of preesterification and transesterification was developed to produce Biodiesel from crude *J. curcas* oil [72]. In this study, a preesterification step was applied to eliminate FFAs by reacting the oil with methanol in the presence of an acid catalyst. The purified oil was further reacted with methanol in presence of an alkali catalyst. Finally, the Biodiesel product was separated from the glycerol byproduct by phase separation. Factors influencing the preesterification and transesterification were also investigated. The authors claimed that the yield of Biodiesel by transesterification was higher than 98% in 20 min using 13% KOH as catalyst and a molar ratio of methanol to oil 6:1 at 64 °C [72].

Recently, a group of Taiwanese scientists developed subcritical hydrolysis and supercritical methylation method for Biodiesel production from *Jatropha* oil [73]. They studied the effects of the reaction temperature, the reaction time and the solvent to feed ratio on free fatty acids in the hydrolyzed oil and fatty acid esters in the methylated oil. This study demonstrated that supercritical methylation preceded by subcritical hydrolysis of the SC–CO_2 oil is a feasible two-step process in producing Biodiesel from powdered *Jatropha* kernels. The purity level of Biodiesel obtained in this method was about 98.5%. The authors reported that the two-step process of hydrolysis and subsequent methylation was amore suitable pathway for producing Biodiesel from *Jatropha* kernel. This study may be of great help to take up future study of carbon dioxide assisted transesterification processes [73].

Various sources of oils were analyzed for their suitability for Biodiesel production including *Jatropha* oil [74]. The oils used were *J. curcas*, neem (*Azadirachta indica*), moringa (*Moringa oleifera*), trisperma (*Aleurites trisperma*), castor beans (*Ricinus communis*) and candlenut (*Aleurites moluccana*). This study proved that among the different oils compared, according to the oil yield and the fatty acid composition of the oil, *Jatropha* was the most promising oil seed for Biodiesel production in Cuba. From this study it is evident that the *J. curcas* seed oil has become an indispensable source for Biodiesel production.

3.3. Enzyme studies

Enzyme studies had been conducted in the seeds of *J. curcas* by Staubmann et al. [75] to improve the oil for Biodiesel. They had extracted two new esterases and a lipase. They had characterized the enzymes, including molecular weight, isoelectric point, optimum pH, optimum temperature, K_m and V_{max}. The lipase hydrolysed both short chain and long chain triglycerides at the same rate but was inactive on alpha-methylbenzyl acetate.

In a recent study, de-Sousaa et al. [76] used lipase isolated from germinating seeds of *J. curcas* for lipid hydrolysis to release the FFA for the subsequent esterification with alcohol. They reported that the lipase isolated from germinating physic nut could be used to hydrolyze a wide range of Biodiesel raw materials (vegetable oils, tallow and Biodiesel waste); of these, soy and physic nut oil showed especially high hydrolysis conversion (97% FFA). The Biodiesel also met the required standards.

An enzymatic process for Biodiesel production using *J. curcas* oil was developed by a team of Japanese scientists [77]. In this study, they compared the efficiency of immobilized-whole cell of *Rhizopus oryzae* and commercial lipases (Novozym 435) for the Biodiesel production from *J. curcas* oil. They also tested different alcohols as a hydroxyl donor and reported that methanolysis of *Jatropha* oil progressed faster than other alcoholysis regardless of lipases used. The maximum methyl esters content in the reaction mixture reached 80% after 60 h using *R. oryzae*, whereas it is 76% after 90 h using Novozym 435. According to this study, both the lipases could be used for repeated batches and both lipases exhibited more than 90% of their initial activities after five cycles. The authors suggested that whole-cell of *R. oryzae*, immobilized, was a promising biocatalyst for producing Biodiesel from oil [77]. These types of enzymatic means of Biodiesel production should be developed in future; being a biocatalyst enzyme can alleviate all environmental related problems encountered in chemical processes of Biodiesel production from *Jatropha* oil.

3.4. Engine performance

A few investigations had studied the performance of engines with *Jatropha* based Biodiesel; these studies are vital since they deal with the end use of Biodiesel. A study was conducted by Reddy et al. [78] at IIT-Madras, India to evaluate the performance of the CI engine fuelled by *Jatropha* oil. This study was undertaken with a view to find out the influence of injection system parameters and in-cylinder swirl level on the performance, emission and combustion characteristics of a *Jatropha* oil-fuelled CI engine. The results obtained were also compared with diesel operation. This study elucidated important parameters affecting the performance of CI engine; it reported that the ignition delay with *Jatropha* oil was always higher than diesel under similar conditions. The emissions with *Jatropha* oil were even lower than diesel indicating the sustainable and eco-friendly applications of *Jatropha* based fuels. This study also proved that the hydrocarbon emission level was 532 ppm with *Jatropha* oil as against 798 ppm with diesel. An important model was also developed at IIT-Madras, India to study the combustion characteristics and the kinetic mechanisms responsible for combustion generated emissions of Biodiesel [79]. Comparisons of the results with those of diesel combustion have been made in few cases. It was observed that the burning rate of Biodiesel was less by about 11% than those for diesel with the same air velocity and sphere size. This type of fundamental studies on engine performances could provide the support for utilizing *Jatropha* based Biodiesel.

A study on the combustion characteristics of *Jatropha* oil droplet at various oil temperatures was conducted by Wardana [80]. Combustion characteristics of *Jatropha* oil have been observed experimentally by igniting the oil droplet of various diameters and temperatures on a junction of a thermocouple. He reported that the *Jatropha* oil droplet performed in two step combustion. Fatty acid was burned in the first step and glycerol was burned in the second step. The onset of microexplosion occurred shortly before the second step combustion and it became more frequent as the oil temperature was increased.

Recently Jindal et al. [81] optimized the engine design parameters viz. compression ratio and injection pressure, for better performance of pure Biodiesel obtained from *Jatropha* oil as methyl ester. They studied the effects of various engine design parameters, compression ratio (CR) and fuel injection pressure (IP) jointly on the performance with regard to fuel consumption, brake thermal efficiency (BTHE) and emissions of CO, CO_2, HC, NOx and smoke opacity. The authors reported that the combined increase of compression ratio and injection pressure increased the BTHE and reduced fuel consumption while having lower emissions. The authors suggested that this study may be of great help in the usage of *J. curcas* Biodiesel in direct injection diesel engines, used extensively for agricultural applications. This type of study will help the growth of sustainable agriculture in developing countries by utilizing the wasteland for *Jatropha* plantation, since this study deals with the application of *Jatropha* oil on agricultural equipments that directly benefit the rural farmers.

4. Conclusion

Fossil fuels pose a great threat to energy security and also adversely affect the environment. The momentum has begun for the (partial) replacement of fossil fuels by biofuels. Edible vegetable oils could not be used for the production of biofuels due to their high cost and direct competition with human consumption. Being a nonedible plant, *Jatropha* has emerged as a unique and indispensable feedstock for Biodiesel production. Enormous efforts have been taken by policy makers and governments for the plantation of *Jatropha* in wastelands and equal attention has been given by the scientists for the optimum utilization of this plant for the Biodiesel production. *Jatropha* research may grow further in the years to come for strengthening energy security and protecting the environment from the negative effects of fossil fuels while helping effectively to utilize the wastelands especially in the developing world.

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